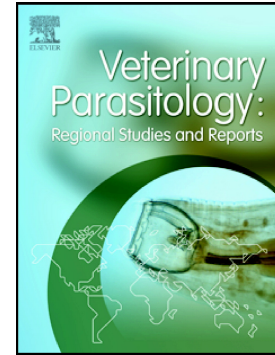


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Use of Molecular Tools for the Diagnosis of Rangeliosis by *Rangelia vitalii* in Argentina: a case report

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Abstract

Vector-borne pathogens are responsible for serious emerging diseases and *Rangelia vitalii*, the etiologic agent of canine rangeliosis, is one of the most pathogenic tick-borne pathogens for dogs in South America. This protozoan is transmitted by the *Amblyomma aureolatum* tick bite and the clinical features associated to the disease are fever, hemolytic anemia, jaundice, hepatosplenomegaly and bleeding from natural orifices, mainly from the ear edge. The reports of canine rangeliosis in Argentina are scarce. In the present study we report the detection of *Rangelia vitalii* in a naturally infected dog from Gualaguay, Entre Ríos, Argentina with history of tick infestation and clinical signs compatible with rangeliosis. An initial blood sample was positive to piroplasms by blood smear examination and the molecular amplification of a fragment of the

18SrRNA gene. Sequencing of the fragment confirmed the pathogen identity. After treatment with imidocarb dipropionate, the clinical signs remitted and the blood smear tested negative.

Key words: Piroplasms, *Rangelia vitalii*, dogs, molecular diagnosis.

Introduction

Rangeliosis is an emerging tick borne disease that affects dogs from Brazil, (Loretto y Barros, 2004; Da silva et al., 2012; Franca et al 2010, 2014) Argentina, (Eiras et al., 2014; Sánchez et al., 2014) Uruguay (Soares et al., 2015, Rivero et al., 2017) and Paraguay (Inácio et al 2017). It is caused by *Rangelia vitalii*, a tick-borne protozoan parasite from the Phylum Apicomplexa that is transmitted by the *Amblyomma aureolatum* tick-bite (Soares et al., 2018). In the life cycle of this parasite, domestic dogs and wild canines act as intermediary hosts. The clinical condition is characterized by fever, hemolytic anemia, jaundice, hepatosplenomegaly as well as spontaneous bleeding from natural orifices in the body, skin and particularly from the ear edge. Rangeliosis is also known as Nambiuuvú, which in Guaraní language means “bleeding ears”. Unlike *Babesia vogeli* and *Babesia gibsoni*, *R. vitalii* merozoites can be localized free at plasma (merozoites type I and II), in red blood cells or monocytes/neutrophils (Loretto & Barros 2005, Soares et al., 2011; Sanchez et al., 2017). This feature facilitates the diagnosis of this parasite infection through blood smear examination. However, diagnosis confirmation could require the use of molecular methods that enable the characterization of the parasitic agent. In Argentina, Rangeliosis has been occasionally reported in Leandro N. Alem, Misiones (Eiras et al., 2014) and in Concordia, Entre Ríos (Sanchez et al., 2014, 2017). The present work aimed to report the use of molecular methods for the diagnosis of Rangeliosis caused by *Rangelia vitalii* in a canine from Gualaguay, Entre Ríos, Argentina.

Case presentation

A 6-year-old male, Jack Russell Terrier mixed breed dog, native from a rural area of Gualaguay, Entre Ríos, Argentina, presented to the veterinary due to two-days history hyperthermia, icteric

mucous membrane, apathy, anorexia and a prolonged bleeding time from the ear edge. The dog had no travel history in recent months but had been infested with ticks. A Complete Blood Count (CBC) and a blood biochemical profile test revealed normochromic, normocytic anemia, thrombocytopenia, leukocytosis, elevated concentrations of urea, and activities of alanine aminotransferase (ALT) and aspartate aminotransferase (AST). In addition, a buffy coat cytology observation was performed and revealed free and intracellular basophilic elliptical structures in erythrocytes and leucocytes that were compatible with *R. vitalii* (Figure 1). In order to rule out the presence of the common tick-borne pathogen *Ehrlichia canis*, an immunochromatography test was performed which resulted negative. An empiric treatment against *R. vitalii* was started with imidocarb dipropionate (6 mg/kg IM) after atropinization (0.05mg/kg SC). Since the patient's clinical condition and the blood test values improved, a second imidocarb dipropionate dose was administered after 14 days of the first one. A control blood smear examination done after treatment resulted negative for *R. vitalii*.

For further diagnostic confirmation, an aliquot of the blood sample was used for the molecular identification of possible pathogens. DNA was extracted from 200 µl of whole blood using a commercial kit (ADN PuriPrep-S; INBIO HGHWAY) and PCR reactions were performed targeting a fragment of the *18SrRNA* (460 bp), common to the order Piroplasmida (Gubbels et al., 1999) and a nested PCR (200 bp) targeting the *p30* gene for *Ehrlichia canis* (Stich et al., 2002). Both positive (*Babesia bovis* strain R1A, and *E. canis*) and negative (pure water) controls were used. An aliquot of 5 µl of the amplified product was analyzed by electrophoresis in 1% agarose gel stained with ethidium bromide. The *E. canis* PCR tested negative but given that the *18SrRNA* fragment common to the order Piroplasmida was amplified, the remaining amplification product was purified and both strands from the amplicon were sequenced and analyzed on an ABI 3500 genetic analyzer. The gene fragment targeted holds a hypervariable region that enables the genus and species identification. The resulting sequence (accession number MT036310) showed 100% identity with sequences from *R. vitalii* deposited in GenBank (<https://www.ncbi.nlm.nih.gov/>). Moreover, a phylogenetic tree was built using the 410 bp sequence obtained and reference sequences from the

order Piroplasmida and the “Maximum Likelihood” algorithm with 1000 bootstrap iterations (Figure 2).

Discussion

Rangeliosis is an emerging infectious disease in our environment that affects dogs causing a severe hemolytic anemia and bleeding, due to endothelial dysfunction and a decrease in platelet count. The present study represents the southernmost report of a *R. vitalii* case in a dog from Argentina in South America.

Amblyomma aureolatum is a competent vector for *R. vitalii*, and transovarial and transstadial transmission has been proved in this vector (Soares et al., 2018). This tick species is distributed in natural areas of the northeast Argentina (Boero JJ, 1957; Ivancovich JC, 1973; Tarragona et al., 2012; Colombo et al., 2016; Nava et al., 2017), including Entre Ríos (Boero JJ, 1954; Guglielmone et al., 2002). Consequently, those dogs that are used as working animals or as hunters in rural areas without preventive acaricidal treatments, are at a higher risk of being bite by the vector (Carini and Maciel, 1914; Loretto and Barros, 2003; Soares et al., 2014; Rivero et al., 2017). Although to date, there are no cases of *R. vitalii* reported in densely populated urban zones, *A. aureolatum* has been described recently in a natural area of Buenos Aires city (Cicuttin et al., 2017).

Different drugs have been described for the treatment of Rangeliosis: imidocarb dipropionate, doxycycline or diminazenol acetate (Loretto and Barros, 2004; Da Silva et al., 2011; Rivero et al., 2017). In the present case, the administration of two doses of imidocarb dipropionate with an interval of 14 days was enough for the remission of the clinical signs, and also a second blood smear performed after treatment resulted negative for this agent. This good response to the drug is in accordance with the treatment reported for “large forms of *Babesia*”, for which a combination of imidocarb dipropionate and atropine is used (Conrad et al., 1991; Baneth G, 2018).

Rhipicephalus sanguineus sensu lato (Debarbora et al., 2011; Nava et al., 2012; Nava et al., 2018; Borrás et al., 2019) is probably the most common tick in dogs in Argentina, and is a vector of many

pathogens, such as *Ehrlichia canis*, *Hepatozoon canis* and *Babesia vogeli* (Silva et al., 1998; Perez Tort et al., 2012; Eiras et al., 2008, 2013). These pathogens cause similar clinical signs and should be considered, either within the differential diagnosis or as co-infections.

Conclusion

There are different species of piroplasms that are circulating among the South American dogs. For this reason, the use of molecular tools allows professionals to get an accurate diagnosis, and at the same time are valuable for the study of epidemiological aspects of the parasite and its vectors. Rangeliosis should be suspected and included within the differential diagnoses in those areas where the *A. aureolatum* tick has been found, and where rural dogs and peri-urban dogs show clinical signs of anemia, fever, and bleeding. Similarly, it is a key point the use of acaricidal products regularly and systematically as a preventive measure to minimize the exposure and risk of infection in dogs, as well as the early suspicion of this pathology by the veterinary community.

Declarations of competing interest:

The authors declare no conflict of interest.

Ethical statement:

This manuscript describes a clinical case. No animal experimentation occurred.

Acknowledgments

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Figures captions

Figure 1. (A) Free basophilic elliptical structures compatibles with *R. vitalii* merozoites type II. Blood smear. May Grunwald – Giemsa. 1000x. (B) Two merozoites within an erythrocyte. Blood smear. May Grunwald – Giemsa. 1000x. (C) Few merozoites within a monocyte. Blood smear. May Grunwald – Giemsa. 1000x

Figure 2. Phylogenetic tree using 410 bp sequences from the *18srRNA* gene of diverse Piroplasmida species and the Maximum Likelihood algorithm with 1.000 bootstrap iterations. A sequence from the *18srRNA* gene from *Hepatozoon canis* was used as an outgroup. The cluster formed by *R. vitalli* reference sequence and the sample sequence is marked with a box

HIGHLIGHTS

- The southernmost case report of *Rangelia vitalii* in a dog from Argentina in South America
- Molecular detection and sequencing of *Rangelia vitalii*
- A good clinical response and blood tested values improvement after treatment with two doses of imidocarb dipropionate with an interval of 14 days.

Journal Pre-proof

Declarations of competing interest:

The authors of “***Use of Molecular Tools for the Diagnosis of Rangeliosis by Rangelia vitalii in Argentina: a case report***” declare no conflict of interest.

Journal Pre-proof

Ethical statement

This manuscript "***Use of Molecular Tools for the Diagnosis of Rangeliosis by Rangelia vitalii in Argentina: a case report***" describes a clinical case. No animal experimentation occurred.

Journal Pre-proof

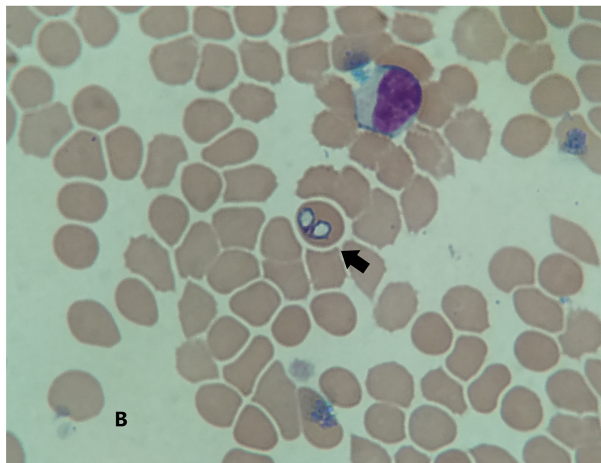
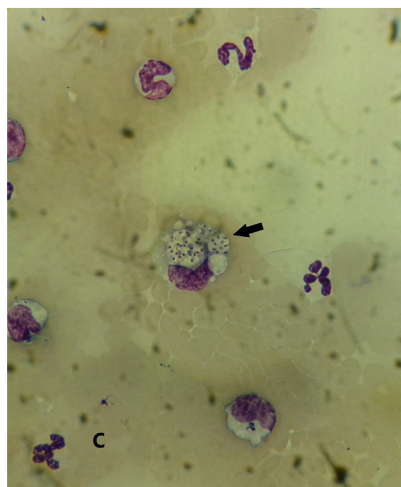
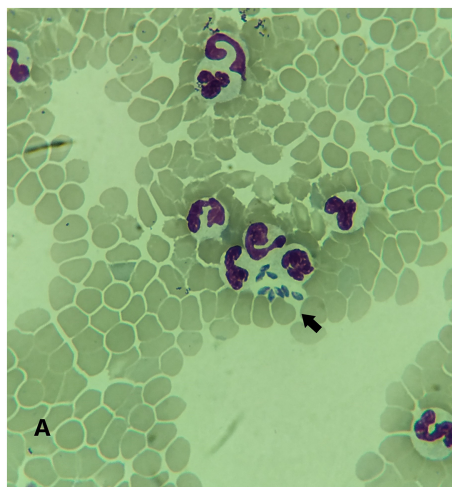


Figure 1

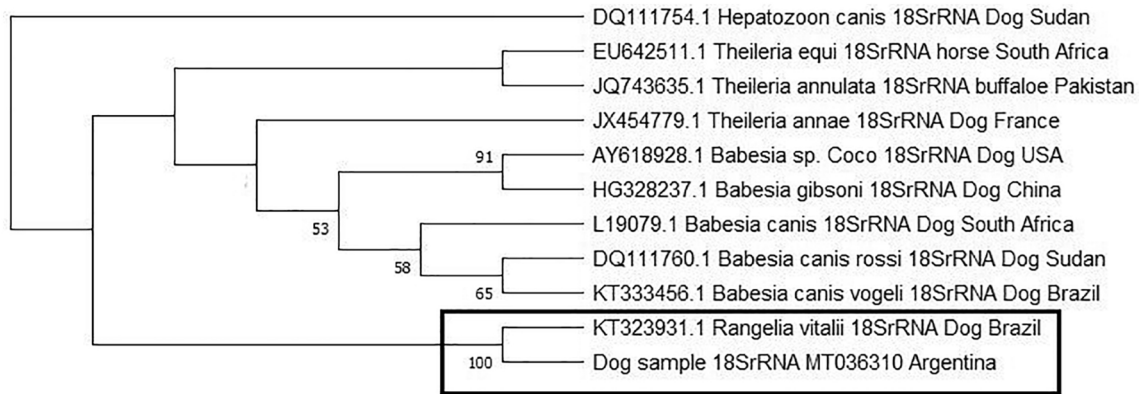


Figure 2